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Radiotherapy-Induced Adverse Skin Reactions

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14. ABSTRACT In response to radiation therapy (RT), many breast cancer patients experience early adverse skin reactions (EASRs) due to inflammation. Therefore, we test alternative medicine with anti-inflammatory properties, Calendula officinalis and Ching Wan Hung, in RT -induced EASRs. We have tested two animal models with two ionizing radiation (IR) sources. First, C57/B L6 mice were used and IR was performed in a clinical facility (Varian 2100C Linear Accelerator). Visible signs of radiation dermatitis, such as blood vessel dilation, erythema, scales, moist desquamation, were observed from day 8 to 20 with a peak on day 16. The mice treated with Calendula Officinalis shown a faster recovery compared to those treated with Ching Wan Hung. Due to new clinical regulation, we had to change IR source. Second, SKH-hr1 hairless mice were used and IR was performed in a research facility (a 100 KV X-ray machine). No significant skin lesions or signs of radiation dermatitis were observed in all groups of animals. Mild skin reactions, such as reddening and scales, were observed in medicine-treated group around days 8~14. In summary, appropriate radiation dosage and topical medicine control will need to be evaluated in future studies as proposed in our no-cost-extension period.				
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Comprehensive and Alternative Medicine in Preventing Radiotherapy-Induced Adverse Skin Reactions

Progress Report

a) INTRODUCTION:

The proposed research has three objectives: (1) To test the molecular mechanisms involved in ionizing radiation (IR)-induced molecular changes; (2) To compare the protective effects of two CAM products, *Calendula officinalis* or Ching Wan Hung, previously used in the treatment of burns; and (3) To identify molecular mechanisms involved in their protective effects, including three targeted pathways, apoptosis, proliferation, and inflammation, as well as genome-wide expression profiles to identify new targets.

b) BODY:

Tissue and blood sample collections: For both control and experimental groups, half of the mice were euthanized at day 5, and the rest half were euthanized at day 16. Blood samples were collected, followed by centrifugation at 3000 rpm for 15 min at 4 °C to separate serum from the blood cells. After centrifugation, the serum was aliquoted and frozen in -80 °C for later ELISA assays. Skin biopsies from the irradiated area on the hind limbs were taken and divided into three parts: 1) For RNA isolation, skin was rinsed quickly in cold PBS, chopped into tiny pieces and immediately stabilized in RNeasy lysis buffer (Qiagen). Total RNA was isolated from approximately 25 mg skin tissue using Illustra RNeasy Mini RNA isolation Kit (GE Healthcare), following the manufacturer's instructions. 2) Part of skin biopsies were fixed in 10% neutral buffered formalin (EMD) for histology analysis and 3) One third of skin biopsies were embedded in Tissue-Tek OCT (Sakura Finetek, CA) and stored in -800C for future immuno-histochemistry studies.

Mice were exposed to ionizing radiation (IR) at the posterior dorsal region to 10 Gy/day for 4 consecutive days. During each irradiation, anesthetized mice were placed on a 1.5 cm thick Lucite plate and irradiated with a 9 MeV Electron Beam irradiator. Hind limbs of mice were exposed to irradiation; the rest of the body was shielded by a 5 mm lead to protect vital organs (**Figure 1**). Total of eight mice were used for each experiment (table). Two groups of control mice (2 mice/group) were treated without or with irradiation. Two groups of experimental mice (2 mice/ group) were treated by irradiation and topical application of either *Calendula officinalis* or *Ching Wan Hung* on both hind limbs at two different time points, immediately prior to irradiation (left leg) or immediately post irradiation (right leg). The physical changes of the mouse skin at the irradiated region were photographed every two days using SONY cybershot camera coupled to a DermLite II pro dermoscopy (3Gen, CA). In the first pilot test, C57/BL6 mice were used. The hairs on the mice hind limbs were removed with Nair (Church & Dwight Co., NJ) two days before the irradiation. The IR was performed in clinical facilities (Varian 2100C Linear Accelerator) of Department of Radiation Oncology, Sylvester Cancer Center, at University of Miami. In the second experiment, SKH-hr1 hairless mice were used. The IR was performed in the research facilities (a 100 KV X-ray machine) of the Department of Radiation Oncology, Dr. Ahmed Mansoor's research lab, Papanicolaou Bldg, room 118, University of Miami



Figure 1: Overview of the Irradiation Setup

1st Trial: Total eight C57/BL6 mice were used for this experiment. The physical appearance of skin at the irradiation affected area was photographed (Figure 2) to evaluate any apparent effects of *Calendula officinalis* or Ching Wan Hung on prevention irradiation-induced early adverse skin reactions (EASRs). Visible signs of radiation dermatitis, such as blood vessel dilation, erythema, scales, moist desquamation, were observed from day 8 to day 20 with a peak on day 16. In general, the signs of irradiation-induced dermatitis were gradually diminished after day 22, and the mice treated with *Calendula officinalis* shown a faster and better recovery compared to those treated with Ching Wan Hung. In both treatment groups, no significant difference was observed between the left and right limbs, indicating the timing to apply the drug, either prior to irradiation or post irradiation, has no apparent difference as shown in this experiment. All experimental mice treated with either topical medicine shown a better recovery compared to the controls.

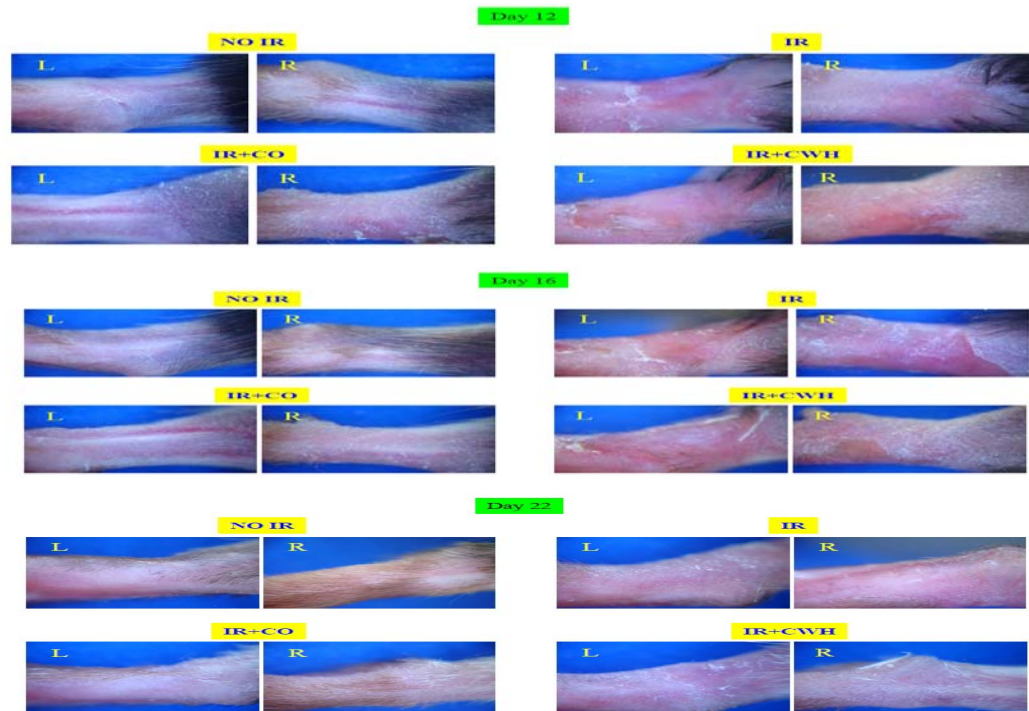


Figure 2: Mice skin images at the irradiated areas. (No IR: no irradiation; IR: irradiation without medical treatment; IR+CO: irradiation plus *Calendula officinalis* treatment; IR+CWH: irradiation plus *Ching Wan Hung* treatment; L: left hind limb; R: right hind limb.)

2nd Trial: Total eight SKH-hr1 hairless mice were used in this experiment. To our surprise, no significant skin lesions or signs of radiation dermatitis were observed in all groups. Mild skin reactions, such as reddening and scales, were observed in medicine-treated group around days 8~14. Much milder and shorter (days 12~14) reactions were observed in the control mice, which received irradiation without any drug treatment. Since no obvious lesions or signs of radiation-dermatitis were observed in this experiment, an appropriate radiation dosage may need to be further determined. Besides, for the topical medicine control, mice treated without irradiation but with the medicines may be included.

c) KEY RESEARCH ACCOMPLISHMENTS:

- Multiple 5 mm thick lead shield specific for mouse animal models were designed and build to prepare mouse for the proposed research.

- Two experiments were conducted with two animal models and two irradiators: sample collections and skin reactions were successfully established. However, another IR source (recently installed at the animal facility) will be used to deliver higher energy source.

d) REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- NIH/NCI Grant Application ID: 1R01CA135288-01A 1 (12/01/2009 to 11/30/2014); Impact of Genomics on Disparities in Breast Cancer Radiosensitivity (priority score: 23 at 6%, within the fundable range)

e) CONCLUSIONS:

- The 5 mm thick lead shield was effective in preventing injury in internal organs from IR. This device can be used for all future animal research.
- Procedures for sample collections and skin reaction evaluations were successfully established. However, another IR source (recently installed at the animal facility) will be used to deliver IR with higher energy source in order to induce skin reactions critical for the proposed research.

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g) APPENDICES:

Not Applicable.